ELSEVIER

Neurobiology of Aging, Vol. 17, No. 1, pp. 25–30, 1996

0197-4580/96 $15.00 + .00

0197-4580(95)00046-1

Scopolamine Differentially Affects Memory of 8- and 16-Month-Old Rats in the Double Y-Maze

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Received 23 March 1994; Revised 7 November 1994; Accepted 14 December 1994

BIGGAN, S. L., J. L. INGLES AND R. J. BENINGER. *Scopolamine differentially affects memory of 8- and 16-month-old rats in the double Y-maze.* NEUROBIOL AGING **17**(1) 25–30, 1996. – The present study investigated the effects of scopolamine on working and reference memory in the same rats at 8 and 16 months of age. Rats were trained in the double Y-maze until a criterion of ≥88% correct was reached on both memory components. Doses of scopolamine (0.1, 0.4, 0.8 mg/kg for rats at 8 months; 0.05, 0.1, 0.4 mg/kg for rats at 16 months) were administered in a counterbalanced order 30 min before test sessions which also included delays of 0, 5, or 30 s prior to both memory components. Results showed that at both ages the 0.1 mg/kg scopolamine dose selectively impaired working memory, whereas higher doses impaired both working and reference memory. Delays selectively decreased working memory choice accuracy and enhanced the effect of scopolamine. Rats at 16 months performed less well on both reference and working memory and showed greater impairments with scopolamine and delays. The present findings support the hypothesis that a decrease in cholinergic neurotransmission contributes to age-related memory deficits.

Age Double Y-maze Reference memory Scopolamine Working memory

MEMORY decline is a common feature of aging. Both aged rats (1,8,21,25,26,28,33,36,39,42) and aged humans (2,14,20) often exhibit impairments in memory. The finding that a decrease in neurochemical markers for cholinergic neurons occurs as a result of aging (2,12,35), and to a greater extent as a result of Alzheimer's disease (11), has prompted much research on the role of cholinergic neurotransmission in memory. Memory impairments have been induced in animals by placing lesions in basal forebrain cholinergic nuclei (5,6,10,16,17,18,23,31,34,37,38,41), and by injections of anticholinergic drugs systemically (3,13,33,39,43) and intracranially (9,15,22,24,40).

In spite of this evidence, the importance of cholinergic neurotransmission in memory has been brought into question in recent years (e.g., ref. 19). Of particular concern has been the observation that basal forebrain injections of the excitotoxins, quisqualic acid, or amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), although producing decreases in cortical cholinergic markers equal to or greater than those produced by excitotoxins such as ibotenic or quinolinic acid, produced significantly less mnemonic impairment (10,16,17,18,31,34,37,38,41). However, in neurochemical experiments it has been found that different excitoxins differentially affected basoamygdaloid cholinergic projections (7), those producing the greatest loss of amygdaloid choline acetyltransferase (e.g., ibotenic acid, quinolinic acid) having been reported to produce greater mnemonic impairments (10,16,17,18,31,34,37,38,41). Furthermore, intra-amygdaloid injections of scopolamine produced a significant impairment of memory (24). Thus, there appears to be continued support for a role of acetylcholine (ACh) in memory.

Testing memory in animals is difficult. In many tasks, a memory impairment can be confounded by age- or drug-related changes in motivation, perception or motor abilities. Recently, we have developed the double Y-maze, a task that in several studies (4,6,24,29,30) has allowed a clear separation of performance based on working and reference memory. The task is unique in that the nonmnemonic demands of the two components are identical; they differ only in the type of memory required to make the choice. Thus, if a treatment is shown to impair selectively the working memory component, it is suggested strongly that such a treatment affected the animals' memory.

Because a reduction in cholinergic neurotransmission has been associated with both the aging process and impaired memory function, it was of interest in the present study to examine the effects of the cholinergic antagonist scopolamine on working and reference memory in the same rats at two different ages (8 and 16 months). Because working and reference memory have been demonstrated to be differentially sensitive to delay effects (6), we also looked at the effects of imposing a delay in each component of the double Y-maze task. It was expected that there

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would be an age-, delay-, and scopolamine-dependent impairment of memory in the double Y-maze, and that the effects of scopolamine and delay on memory would be greater in the rats at 16 months.

METHOD

Treatment of the rats in the present study was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care, and relevant University policy and was approved by the Queen's University Animal Care Committee.

Subjects

Eleven male Wistar rats (Charles River, Canada), arriving at approximately 2 months of age, weighed between 225–250 g at the start of the study. All rats were maintained individually in a temperature-controlled environment (approximately 21°C) with a 12L:12D cycle (lights on at 0700 h). Water was available in the home cages at all times. During training and testing, weights were reduced to 80% of their free-feeding level by daily feeding with measured rations. Rats were tested at both 8 and 16 months.

Apparatus

The double Y-maze (see Fig. 1) was elevated 76 cm above the floor and consisted of a centre stem (55 cm long and 15 cm wide) with two arms extending from each end of the stem at an angle of 120°. Removable wooden barriers could be inserted at the end of each arm and in the middle of the stem to provide 15-cm compartments. The maze walls (26 cm high) and barriers were painted light grey. The floor consisted of steel grids spaced approximately 1 cm apart except at the stem-arm junctions where there were triangular pieces of Plexiglas. Plastic food cups were located in the centre of the goal box adjacent to the end wall of each arm and in the centre of the stem adjacent to the second barrier. Froot Loops cereal was used as a reward and pieces of the cereal were scattered under the grid floor to mask possible reward odor cues. Testing was carried out in a small room in which several visually distinct cues (e.g., experimenter, lights, door, window) were within sight of the rats in the maze.

Procedure

Training. Rats were first tested at 8 mo and then retrained and retested according to the same procedure at 16 months. Food deprivation began 5 days before the start of each training period. Rats were not food deprived in the time between the end of the first test period and the start of retraining at 16 months. During food deprivation the rats were fed their ration of chow plus, for the first 5 days, a small quantity (2 g) of Froot Loops cereal in their home cages. Pieces of the cereal were subsequently used as food reward in the double Y-maze task.

Rats were given 5 days of habituation to the double Y-maze during which they were free to move throughout the maze for a 5-min period. During habituation, several food cups containing Froot Loops cereal were placed throughout the maze. Following this period, the rats were given one training session of 12 trials per day, 7 days per week. Each trial began by placing the rat in one of the end arms of the first "Y". Choice of the start location varied randomly with the condition that no more than half of the trials per day were given from the same side. The barrier was removed and the rat was rewarded for going down the stem, the distal end of which was blocked by a removable barrier (see Fig. 1). Upon entering the region located in the middle of the stem, a second barrier was dropped into place behind the rat preventing reentry into the first "Y". The barrier in front then was removed to allow access into the second "Y". The rat continued up the stem and was rewarded again for entry into the appropriate goal box of the second "Y".

The correct choices required the use of both working and reference memory. The reference memory component was to always go down the stem in the first "Y" and enter the start box in the middle of the stem regardless of which end arm of the first "Y" was the starting position. The correct working memory choice was to enter the arm of the second "Y" on the side of the maze diagonally opposite the side of the first "Y" from which that particular trial had begun (for half of the rats) or on the same side as the start location (for the other half of the rats).

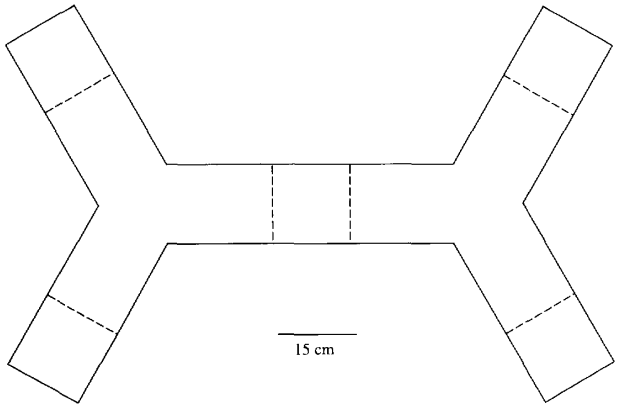

FIG. 1. Double Y-maze. Broken lines indicate manually operated barriers which could be used to restrict access to each part of the maze. Rats made the reference memory choice in the Y-maze to the right and the working memory choice was made in the Y-maze to the left.

If the rat made an incorrect working memory choice, it was removed from the maze and the trial ended. When a rat made a reference memory error it was removed from the maze before it could make a working memory choice; therefore, although each rat received 12 reference memory trials per day, at times rats received fewer than 12 working memory trials. A working or reference memory choice was defined to have taken place when the hind legs crossed onto the grid floor of the arm.

The number correct reference and working memory choices were recorded daily. Training continued at 12 trials per day until the rats reached a criterion of at least 88% (32/36) choice accuracy on both memory components over a 3-day block.

Drug testing. This phase consisted of five 3-day blocks of drug treatment with one testing session per day. Each treatment block was followed by a minimum of 3 days of training to reestablish criterion level of performance. Each session of the first and fifth treatment blocks was preceded 30 min by an IP injection of 0.9% saline (1 ml/kg). Sessions of the second, third, and fourth treatment blocks were preceded 30 min by an IP injection (1 ml/kg) of scopolamine hydrobromide (Sigma Chemicals). Scopolamine was dissolved in 0.9% saline at concentrations of 0.1, 0.4, and 0.8 mg/ml for testing at 8 months, and at concentrations of 0.05, 0.1 and 0.4 mg/ml for testing at 16 months. Pilot studies had shown that the reduced doses for the rats at 16 months produced memory impairments similar to those seen with the higher doses administered at 8 months. At both testing times rats were randomly assigned to one of the six possible dosage orders.

The task for drug testing remained the same as in training trials, the only difference being the use of variable delays (0, 5, and 30 s). The reference memory component of each trial began by placing the rat into one of the end arms of the first "Y" with the barrier in place for the appropriate delay. Once the rat entered the centre region of the stem, the barrier behind the rat was dropped into place but the barrier in front was not removed until after the appropriate delay. The rat then was released into the second "Y" to make the working memory choice. The delay prior to each choice was the same for any individual trial. Rats were given 12 trials per day, with 4 trials at each delay. As in the training procedure, the number correct working and reference memory trials were recorded.

RESULTS

The mean (± SEM) number of correct responses as a function of delay and treatment condition are shown for the reference and working memory components of the task in Fig. 2 and Fig. 3, respectively. Analyses of variance (ANOVAs) comparing the two saline treatment blocks across delays showed no significant difference for both the reference and working memory components, $p > 0.05$, at either age. Thus, subsequent analyses used the number of correct responses averaged across the two saline blocks.

Reference memory was impaired in a dose-dependent manner by scopolamine in rats at both 8 and 16 months (Fig. 2). In rats at 8 months there was little effect of the 0.1 mg/kg dose whereas 0.4 and 0.8 mg/kg dose-dependently produced large decreases in the number of correct responses. In rats at 16 months there was little effect of the 0.05 and 0.1 mg/kg doses whereas the 0.4 mg/kg dose produced a large decrease in the number of correct responses. There appeared to be no consistent effect of delay at either age.

This description of the data was supported by statistical analyses. Reference memory scores from rats at 8 and 16 months

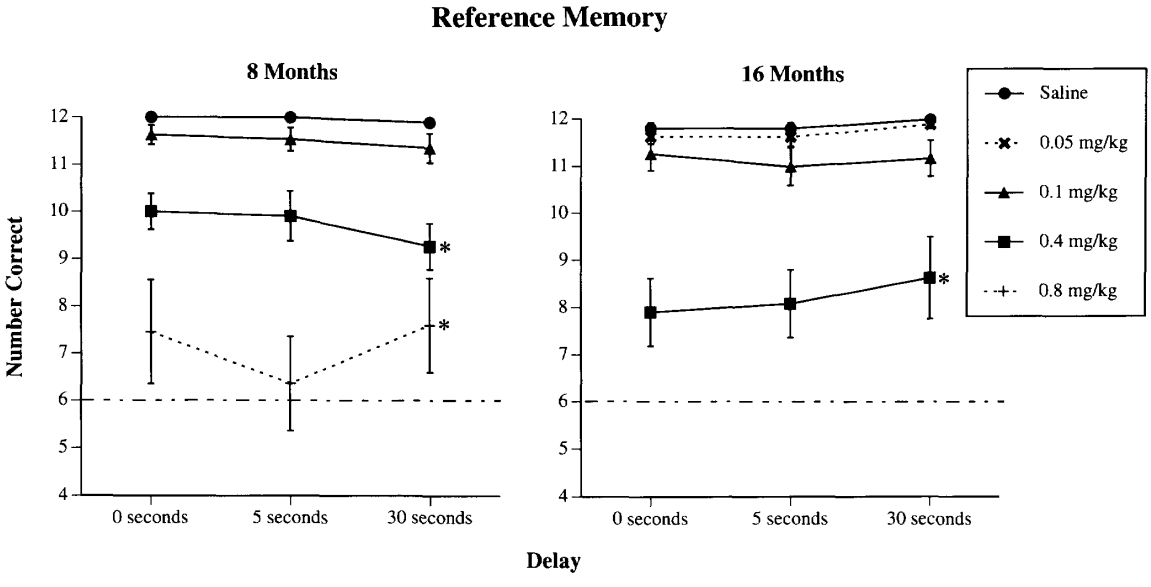

FIG. 2. Mean (± SEM) number of correct reference memory choices at 0, 5, and 30 s delays following scopolamine or saline administration for rats at 8 and 16 months. Solid lines indicate treatments received by rats at both ages whereas the broken lines indicate treatments received at only one age. The horizontal broken line at 6 correct choices indicates chance performance. ANOVAs revealed a significant treatment effect at both ages ($p < 0.005$). There appeared to be little effect of delay at either age. *Post hoc tests showed that for the rats of 8 months the 0.4 and 0.8 mg/kg doses, averaged over delay, differed from saline; at 16 months the 0.4 mg/kg dose differed from saline.

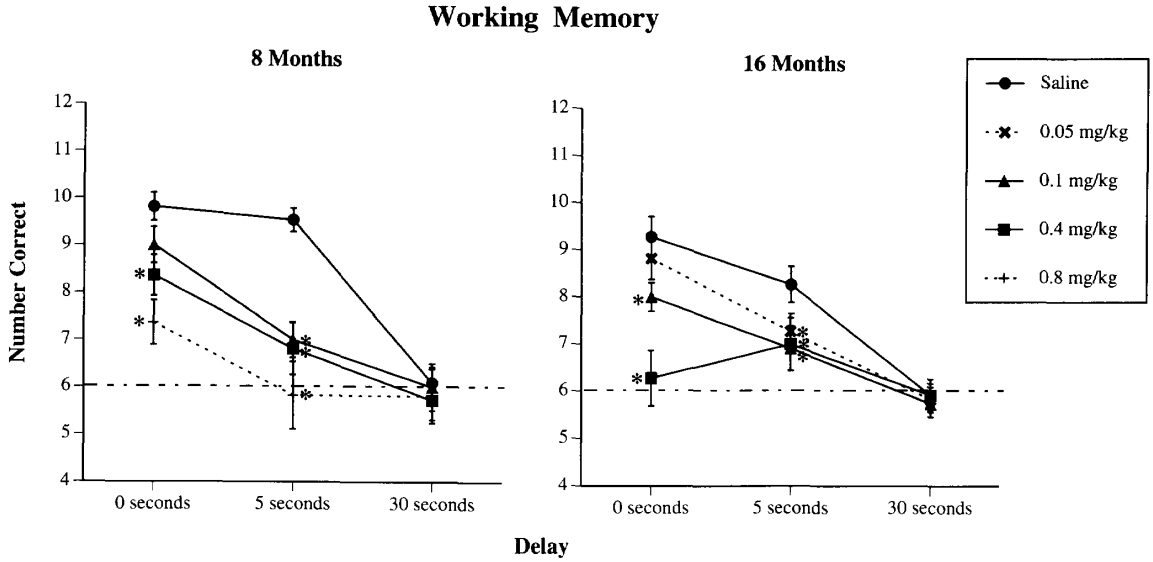

FIG. 3. Mean (± SEM) number of correct working memory choices at 0, 5, and 30 s delays following scopolamine or saline administration for rats at 8 and 16 months. Solid lines indicate treatments received by rats at both ages while the broken lines indicate treatments received at only one age. The horizontal broken line at 6 correct choices indicates chance performance. ANOVAs revealed a significant treatment effect, a delay effect and a treatment by delay interaction for rats at both ages ($p < 0.05$). An age × treatment × delay interaction ($p < 0.05$) shows that the effects of scopolamine depended both on age and delay. *Different from saline in Dunnett's test ($p < 0.05$).

were subjected to two-way ANOVAs. The Greenhouse-Geisser adjusted degrees of freedom for repeated measures were employed, but for clarity the nonadjusted degrees of freedom are presented in the text. The variables analysed were treatment (saline, 3 age-specific scopolamine doses) and delay (0, 5, 30 s). For the rats at 8 months the ANOVA revealed a significant treatment effect, $F(3, 30) = 23.0$, $p < 0.005$, but no delay effect, $F(2, 20) = 1.97$, $p > 0.05$, or treatment by delay interaction, $F(6, 60) = 1.70$, $p > 0.05$. Posthoc comparisons, using Dunnett's t tests, of number correct reference memory responses averaged over the 3 delays showed that both the 0.4 and 0.8 mg/kg doses differed significantly from saline, $p < 0.05$. Similarly, the ANOVA with the rats at 16 months yielded a significant treatment effect, $F(3, 30) = 17.43$, $p < 0.005$, but no delay effect, $F(2, 20) = 2.61$, $p > 0.05$, or treatment by delay interaction $F(6, 60) = 0.66$, $p > 0.05$. Dunnett's posthoc tests, comparing each dose to saline and averaging across the delays, showed that the 0.4 mg/kg dose produced significantly less correct reference memory responses than saline, $p < 0.05$.

In contrast, working memory was impaired in rats at 8 and 16 months in both a dose- and delay-dependent manner. The effect of dose differed depending on the delay (Fig. 3). This was confirmed by the ANOVAs of working memory results from rats at 8 and 16 months. For the rats at 8 months the ANOVA yielded a significant treatment effect, $F(3, 30) = 10.54$, $p < 0.0001$, a significant effect of delay, $F(2, 20) = 48.50$, $p < 0.0001$, and a treatment by delay interaction, $F(6, 60) = 2.68$, $p < 0.05$. In a breakdown of the interaction, a significant treatment effect was shown at both the 0-s delay, $F(3, 60) = 6.44$, $p < 0.005$, and at the 5-s delay, $F(3, 60) = 10.0$, $p < 0.005$. Dunnett's posthoc tests, comparing each dose to saline, showed that at the 0-s delay both the 0.4 and 0.8 mg/kg doses produced significantly less correct working memory responses than saline, $p < 0.05$; however, at the 5-s delay all three doses differed significantly from saline, $p < 0.05$.

Similarly, the working memory ANOVA with the rats at 16 months yielded a significant treatment effect, $F(3, 30) = 5.65$, $p < 0.01$, a delay effect, $F(2, 20) = 42.21$, $p < 0.001$, and a treatment by delay interaction $F(6, 60) = 4.02$, $p < 0.05$. In a breakdown of the interaction, a significant treatment effect was shown at both the 0-s delay, $F(3, 60) = 9.32$, $p < 0.005$, and at the 5-s delay, $F(3, 60) = 6.42$, $p < 0.005$. Dunnett's posthoc tests, comparing each dose to saline, showed that at the 0-s delay both the 0.1 and 0.4 mg/kg doses produced significantly less correct working memory responses than saline, $p < 0.05$; however, at the 5-s delay all three doses differed significantly from saline, $p < 0.05$. For the rats at both 8 and 16 months, there was no treatment effect at the 30-s delay; all treatments (including saline) produced chance performance, suggesting great task-difficulty at this delay.

The analyses of working memory showed that at the 0-s delay the 0.1 mg/kg dose significantly impaired the rats at 16 months but not at 8 months; the 0.4 mg/kg dose impaired both ages. At the 5-s delay both age groups were impaired at both the 0.1 and 0.4 mg/kg doses. This pattern of results suggested an interaction of age, treatment, and delay. Thus, a three-way ANOVA with variables of age (8, 16 months), treatment (saline, 0.1, 0.4 mg/kg), and delay (0, 5 s) was conducted. The 30-s delay was not included as both age groups at all doses performed at chance. As expected, the analysis yielded a significant effect of age, $F(1, 10) = 7.87$, $p < 0.05$, an age by delay interaction, $F(2, 20) = 5.40$, $p < 0.05$, and an age by treatment by delay interaction, $F(2, 20) = 4.19$, $p < 0.05$. Thus, the effects of treatment with scopolamine depended on age and delay as described above. The

three-way ANOVA of reference memory results revealed a significant age effect, $F(1, 10) = 7.97$, $p < 0.05$, while the three-way interaction was not significant, $F(4, 40) = 1.71$, $p > 0.05$.

DISCUSSION

Results showed that the cholinergic antagonist, scopolamine, when administered to rats at both 8 and 16 months produced a dose-dependent impairment of working and reference memory in the double Y-maze. However, the two types of memory were differentially sensitive to scopolamine. While the 0.4 and 0.8 mg/kg doses impaired both working and reference memory, a dose of 0.1 mg/kg selectively impaired working memory. Because the double Y-maze places equal motor, sensory, and motivational demands on the two components of the task, it is unlikely that this dissociation would be produced by a non-mnemonic behavioural deficit, because such an impairment would be expected to affect both task components equally. This implies that at the 0.1 mg/kg dose the rats suffered a specific impairment in memory.

One of the defining characteristics of working versus reference memory is their differential susceptibility to delay effects (6). The observation of a significant delay effect on the working but not reference memory component of the double Y-maze task supports the use of this paradigm for assessing these two types of memory. Furthermore, results showed that delay increased the detrimental effects of scopolamine on working memory performance. These observations suggest a dichotomous classification of memory type with differential susceptibility to cholinergic manipulation and delay.

Results are consistent with previous reports (1,8,21,25,26,28, 33,36,39,42) showing that memory is impaired in older rats when compared to younger rats. When results were averaged across treatment and delay, rats at 16 months displayed greater working and reference memory impairments than rats at 8 months. Furthermore, examination of Fig. 2 and Fig. 3 shows that rats at 16 months had greater deficits in working memory than reference memory in the saline condition, ruling out the possibility that double Y-maze performance was affected by changes in nonmnemonic functions associated with aging. The age × delay and the age × treatment × delay interactions show that scopolamine and delay had a greater effect in the rats at 16 months. The increased sensitivity to delay is seen most clearly in the saline treatment at the 5-s delay and suggests that working memory function declines as age increases. This is consistent with previous studies (1,26,36,42) that have shown selective working memory deficits in aged rats. The increased effect of scopolamine in the rats at 16 months is seen most clearly at the 0-s delay where the 0.1 mg/kg dose had no significant effect at 8 months but did at 16 months; also, the effect of the 0.4 mg/kg dose was greater at the 0-s delay in 16-month-old rats. These results suggest that susceptibility to the memory impairing effects of cholinergic blockade increases with age as has been reported previously in rats (33) and humans (20). One previous study (39) failed to observe increased sensitivity to scopolamine in aged rats in a complex maze but, as the authors themselves suggest, the doses of scopolamine (0.5 and 0.75 mg/kg) may have been too high. The present data do not permit an evaluation of the possibility that the enhanced susceptibility to scopolamine in aged rats is attributable to age differences in pharmacokinetics. Our results with scopolamine support previous reports that suggest that decreases in cholinergic neurotransmission may contribute to age-related memory deficits (2,14,20,25,28,33,35,36).

The present observation of differential effects of age (saline condition) and scopolamine (0.1 mg/kg) on working and reference memory suggests that the double Y-maze is a task that allows for a relatively specific assessment of memory although it is not possible to determine from the present data what type of memory, e.g., spatial, nonspatial, the rats were using. It is possible that an age- or drug-induced motor hypoactivity might have differentially affected working and reference memory; working memory might have been selectively impaired because of the lengthened retention interval. Although latency data were not collected in the present experiment, the effects of scopolamine on working memory did not appear to result from a confounding effect on motor behaviour. Scopolamine administration appeared to have a dose-dependent excitatory effect on motor behaviour. This effect is consistent with previous reports that both systemic and central scopolamine induced transient hyperactivity (24,27,32).

Another possibility is that the differential effects of age (saline condition) and scopolamine (0.1 mg/kg) on working and reference memory were due to the relative difficulty of the two components since acquisition of the reference memory component was more rapid than acquisition of the working memory component. This possibility cannot be ruled out; however, it is noteworthy that Hepler et al. (23), specifically equating the working and reference memory demands of a T-maze task, found a selective impairment of working memory following ibotenic acid lesions of the basal forebrain. This study shows that differential difficulty of working and reference memory tasks does not provide an adequate explanation for dissociations between these two types of memory. Thus, it appears that the double Y-maze is a valuable tool for assessing the mnemonic effects of age and pharmacological manipulations.

The present finding that scopolamine differentially affected memory in rats at 8 and 16 mo is in good agreement with an extensive literature implicating ACh in age-related memory loss (2,11,12,14,20,25,28,33,35,36). This literature includes the finding that patients with Alzheimer's disease, initially characterized by an impairment of memory for recent events (working memory), suffer from a loss of basal forebrain cholinergic cells (11). Undoubtedly, the cholinergic hypothesis represents an oversimplified concept of the neural basis of dementia; however, the present results suggest that a rejection of a role for ACh in memory may not be justified.

ACKNOWLEDGEMENTS

Funded by a grant from the Natural Sciences and Engineering Research Council of Canda to Richard J. Beninger.

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